ON THE DIFFERENTIATION OF THE ORGANS DURING THE LIFE CYCLE PROBOSCIDACTYLA SP. WHICH IS ASSOCIATED WITH THE TUBE OF PSEUDOPOTAMILLA OCCELATA

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ON THE DIFFERENTIATION OF THE ORGANS DURING THE LIFE CYCLE *PROBOSCIDACTYLA* SP. WHICH IS ASSOCIATED WITH THE TUBE OF *PSEUDOPOTAMILLA OCCELATA*[1)]

By

YOSHIKO KAKINUMA

柿沼　好子

Marine Biological Station, Tôhoku University, Asamushi, Aomori City

Many hydroids are associated with molluscs as gastropods, bivalves, scaphopods and other animals. One of the hydroids the colony of *Proboscidactyla* sp. has a symbiotic association with *Pseudopotamilla occelata* a tube living maine worm. *Pr.* sp., perhaps *Pr. flavicirrata,* can make a colony on the worm tube with the living worm. Nothing is known about the means of this specific obligatory association. Hirai (1960) described some interesting experiments on what happens to the *Proboscidactyla* when the worm dies or is removed from the tube. Hirai found the polyps and reproductive polyp (gastrozooid) degenerated and to have disappeared in about two days when the worm was removed. At the same time the stolonic network became activated and the club-like branches of the stolon (dactylozooid) began to increase in number and in length, entangling each other. They were capable of attachment to glass and may be regarded as asexual bodies. In the second experiment, Hirai removed a worm tube, placed it on an open glass tube of suitable diameter and tied the old tube with its stolons alongside. Again the stolons became reactivated and after about ten days the worm had secreted a new tube. By this time a few broken pieces of stolon had found their way onto the glass and one stolon from the original tube had grown up on the outside of the glass tube and down on the inside to make contact with the worm's newly secreted tube. A few days sufficed for a young colony with polyps to be formed. In 1969, I made further experimental investigations to know the reasons for the differentiation of the gastrozooid of *Pr.* sp. The details of those experiments on the symbiotic relations between the differentiation of *Proboscidactyla* from *Pseudopotamilla* are presented in this paper.

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MATERIALS AND METHODS

Proboscidactyla sp., perhaps *Pr. flavicirrata*, is commonly found on the worm tube of *Pseudopotamilla occelata* in the shallow coastal waters in the neighbourhood of the Asamushi Marine Biological Station. The materials used in this study were collected by breaking down the rocks of the shore in front of the station and at Tsuchiya near the station. In the laboratory, the materials of *Proboscidactyla* and *Pseudopotamilla* were kept in running sea water for observations of the life cycles. On the other hand, the materials of each experiment were reared in sea water at 20°C, changing the water every day. Both *Proboscidactyla* and *Pseudopotamilla* could survive on the mush of the larva of the brine shrimp and fish which were used for their foods.

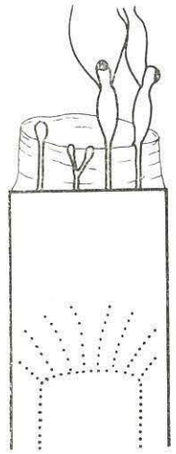

Fig. 1. Colony formation on the new tube regenerated from the original worm tube which was cut off at its edge.

RESULTS

Outline of life cycle of the Proboscidactyla sp. (Pl. II).

The colony of Pr. sp. was found on the upper part of the worm tube of a Polychaeta *Pseudopotamilla occelata* throughout the year in the field. Especially the colony with many polyps of this species was found from spring to autum. Medusa formation was found about from late May to early October. Medusae buds were visible in the middle part of the gonozooid as a transparent glove about 50 μ in diameter in a whorl. In the laboratory the medusae buds grew completely and liberated from the polyp about ten days after the buds appeared, at 20°C room temperature. Young medusa with six radial canals which just escaped from the gonozooid, 0.5 mm in height and 0.7 mm in width. First medusa which liberated from the gonozooid have six radial canals and the number of the radial canals decreased to five, four, gradually in order of liberation of medusa.

Regeneration of polyp (gastrozooid) from the worm tube which was cut off at its edge or rim.

The worm tube of *Ps. occelata* was removed by cutting at its edge or rim part. *Ps. occelata* secreted the substance to regenerate the removed part of the tube within half an hour after the operation. The worm kept moving in and out from this tube and made a new thin transparent tube at its rim. The stolons of the cut end of the colony of *Pr.* sp. were closed up completely in about three

Fig. 2. Gonozooid which arised on the entangling stolon on the glass bottom.

hours after the operation. The stolons elongated on the new tube parallel to the tube axis and progressed toward the new rim of the tube. The tips of the stolons became club-like in about 12 hours after the operation and the polyp rudi-

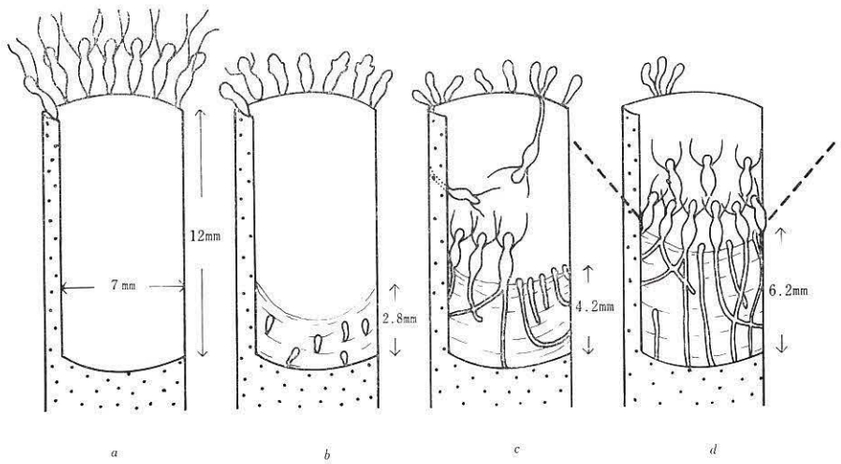

Fig. 3. Reformation of the colony on the newly formed worm tube.
a. Worm tube was cut off in square shape.
b. On the third day after the operation.
c. On the fifth day after the operation.
d. On the eight day after the operation, the colony was reformed completely in this stage.

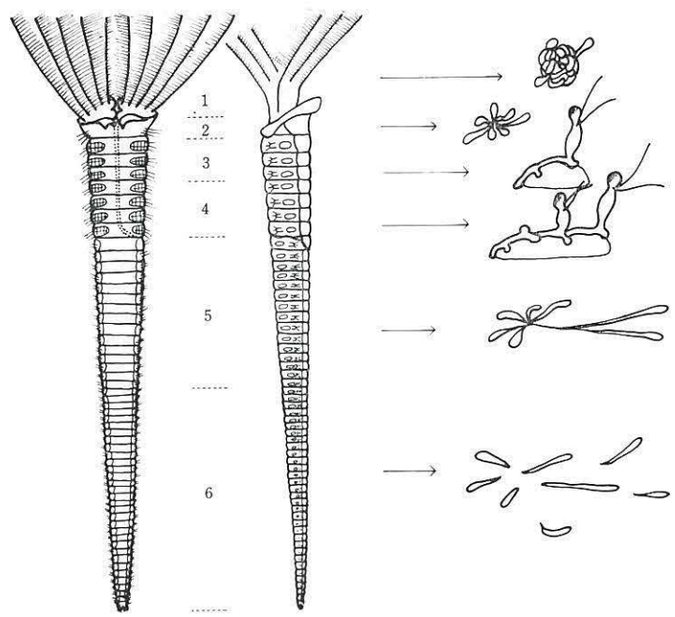

Fig. 4. Regeneration of the polyp from the stolon which touched directly the isolated worm body.

Fig. 5. Differentiation of the polyp on the mass of secreted mucus from the worm trunk.

ments were developed at the tips of the stolons within a day. On the second day, the polyp rudiments became tumbler-like in form, forming the distal part with a head and a mouth, and the proximal part with a column at first. Then the tentacle rudiment appeared as double knobs and elongated rapidly. A whorl colony of the polyps of *Pr.* sp. appeared on the new rim of the worm tube within two days (Fig. 1).

On the other hand, the cut ends of the stolon of the removed piece of the tube closed up completely in about three hours after the operation and formed club-like stolons at the cut end. These were the rudiments of the regenerating stolon in about six hours after the operation. The regenerated new stolons attached on the bottom of the petri-dish and began to elongate, and expanded in various directions. At this time, the initial polyps began to be absorbed in order from the head of polyps, then the column and at last the tentacles. On the second day, several stolons elongated entangling and overlapped each other, and a gonozooid appeared on the top of the elongated stolons. On the third day, the medusa buds developed one after another at the middle part of a gonozooid. The medusa buds grew into the young medusa within ten days and were liberated from a gonozooid (Fig. 2).

Reformation of the colony in Proboscidactyla sp. (Fig. 3).

A part of the edge of the worm tube of *Ps. occelata* was cut in square shape and

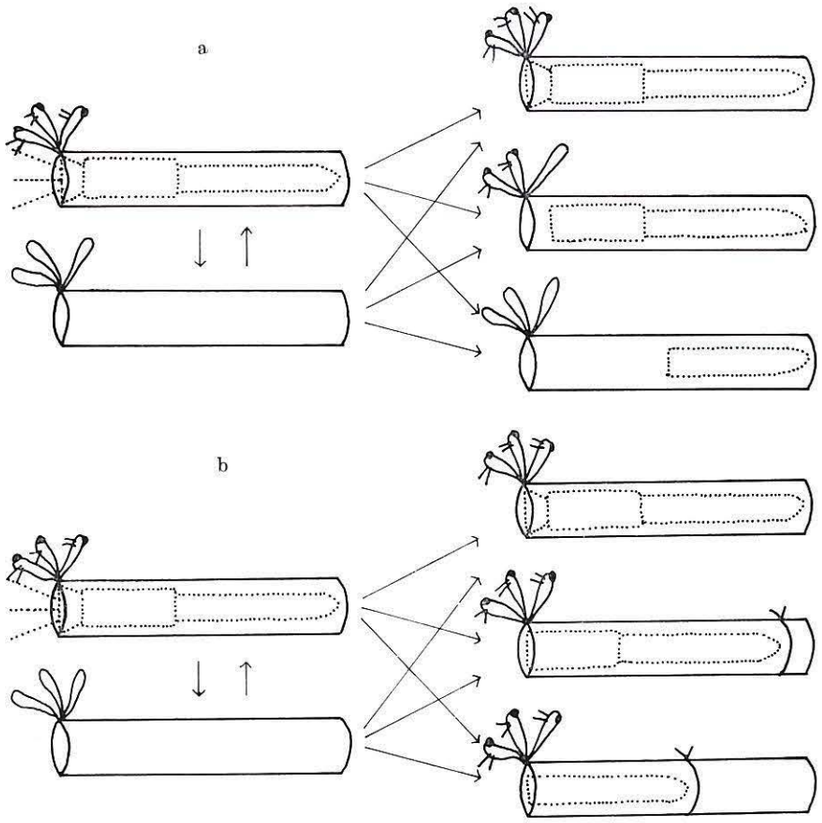

Fig. 6. The differentiation of the polyp at the edge of the tube with the isolated worm body.
a. Polyps at the edge of the tube with isolated pieces of the worm body which were kept free in the tube.
b. Polyps were differentiated from the stolon which kept close contact with the cut pieces of the worm body in the tube.

removed. In such case, the distal part of the worm tube was cut off and was removed with the colony of 12 mm in height and 7 mm in width. The worm escaped into the deeper part of the tube in about a half an hour after the oparation (Fig. 3 a). Then it began move and the worm tentacle was observed coming out and in from the deep cut end, but the tentacle did not reach the edge of the original tube. The worm secreted a new thin transparent tube of about 2.8 mm in height on the upper part of the cut end within two days. (Fig. 3 b). Pieces of the stolon gathered on the newly secreted tube. At this time, the most of the original polyps degenerated to the club-like stolon. On the fifth day, the new tube became about 4.2 mm in height and the stolons increased in number and

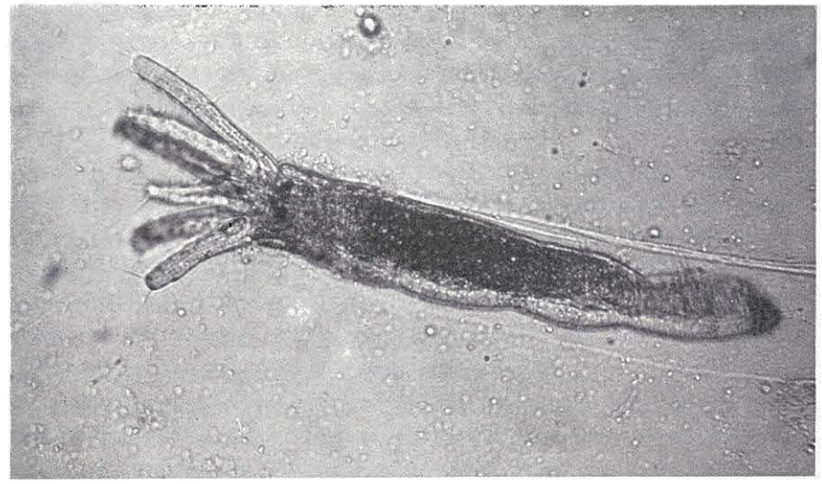

Fig. 7. Young larva of *Ps. occelata* with delicate tube.

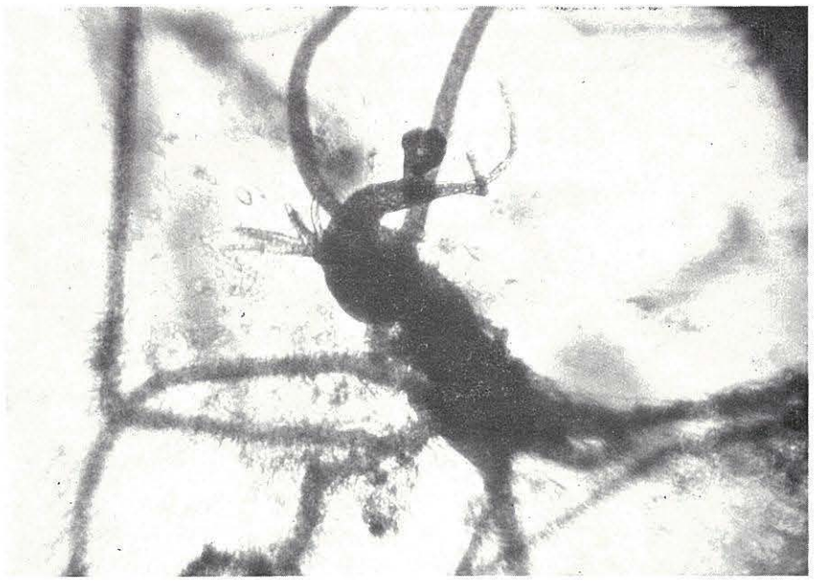

Fig. 8. Differentiation of the polyp on the young larva of *Ps. occelata*.

elongated parallel to the tube axis. The tips of several stolons of the new tube differentiated to the polyp. One or two stolons with the initial polyps of the original tube were elongated along the inside on the original tube and in this case the polyps did not disappear (Fig. 3 c). On the eighth day, the new tube became about 6.2 mm in height and then the worm stopped further secretion, and the rim

became the edge of the new tube. Then the worm kept protruding the tentacle from the mouth of the new tube. The new colony of polyps in circle arrangement were formed again at the new edge of the tube by the newly appeared polyps with the initial polyps which were turned into the inner new cricle of the new tube (Fig. 3 d).

Differentiation of the polyp when the stolon touched directly with the isolated worm pieces of the body of the worm in Ps. occelata.

The body of the worm was separated into six parts (Fig. 4). The first piece was the tentacle, the second the collar, the third and fourth the trunk, and fifth and six pieses were the tail. When the cut end of the isolated pieces of the worm closed up, each piece were wound by the long stolons of *Pr. sp.* respectively.

Stolons which were wound around the tentacles: The stolons kept touching the tentacles which kept active movement continually. After two days, the stolon gradually became a glove mass. The activation of the tentacle movement gradually became weak day by day, and finally the tentacle movement stopped completely in about 10 days after the operation. No differentiation of the organs, gastrozooid or gonozooid, from the stolon were observed during this experiment.

Stolons which were wound around the collar: The collar was not regenerated and the tissue of the collar became disfigured day by day, and in about seven days after the operation it disfigured completely. The stolon removed from the collar became elongated after placed far away on the glass.

Stolons which were wound around the trunk: The isolated piece of the trunk secreted tube substance on its ventral part, keeping expansion and contraction. The substance made a new transparent thin tube membrane after 20 minutes of secretion and the membrane accumulated and became an opaque lump on the upper part of the trunk. After a day, the stolons were found swarming about the lump, and then elongated over the lump. On the third day, a polyp appeared on the tip of the stolon and grew in the direction of elongation of the stolon. The polyp appeared on the tube lump which kept close contact with the cave at the segment of the trunk. This polyp was removed from the worm trunk on the elongation of the stolon and disappeared. In a case which was observed on the trunk with tentacle and collar part, two or three polyps appeared on each one of the stolon tips within two days showing the same course in development as mentioned above (Fig. 5).

Stolons which were wound around the tail: The isolated tail part secreted smaller quantity of tube substance than that of the trunk part, and a transparent thin membrane was observed after three hours of secretion. The membrane was soon released from the tail with the stolon attached on the membrane. No differentiation of the organs from the stolon were observed in this experiment.

Polyp which was kept at edge of the tube with the cut and isolated pieces of the worm bodies in the tube.

The worm bodies were removed from their tube, then some parts of the body were isolated and the piesces were placed respectively in the tubes with the polyps after the cut ends of the worms were healed (Fig. 6).

In the tube with the worm body without the tentacle, the polyps appeared normal. The begining of degeneration in about 30 percent of the polyps were observed without the collar and tentacles.

The degeneration of the number of polyps increased according to the cases of removal of the upper parts of the worm body as shown in Fig. 6.

In the next experiment, when the worm bodies were removed from the tube the polyps degenerated to the stolons within about two days. In those tubes with degenerated polyp, the isolated part of the worm bodies were replaced. In this experiment, some regeneration of polyps from the degenerated stolons were visible, and distinct regeneration was observed only in the tubes in which the whole worm body was replaced. But those result mentioned above depended upon the separation of the rim of the tube with the isolated peices of the worm bodies in the tube as was shown in the figures. Then the pieces of the worm bodies kept close contact with the rim of the tube by closing the tube at the posterior end of the piece with a thread. In this case the polyp did not degenerete, and on the other hand, the polyp which degenerated to stolon regenerated in every case of the piecs of the worm body, though less regeneration was observed in the tail part.

Differentiation of polyp from the stolon which was adheared on the tube of larva of Pr. sp.

In February 1969, the development from the eggs to the larvae of *Ps. occelata* in petri-dishes at about 15°C–18°C in the laboratory was observed. The stolons of *Pr. sp.* were scattered around the polychaet-larvae eight days after spawning of *Ps. occelata*. In this stage, a thin transparent tube was observed along the larva (Fig. 7). The stolons elongated in various directions on the bottom of the dish and when the stolons touched the fine tube of *Ps. occelata*, they elongated towards the opening of the worm tube and the polyp of normal size was differentiated (Fig. 8). The young larva seemed to go down under the weight of the polyp, but the growth of the larva was not disturbed by the polyp. On the other hand some of the polyps appeared on the tube at the end of the tail of the larva but they disappeared within about two days without keeping the polyp for a long days.

CONSIDERATION

A hydrozoan *Proboscidactyla* sp. lives comensal upon the tube of *Pseudopotamilla occerata*. In 1960, from his experimental investigation Hirai described that the differentiation of this species greatly depend on the external factors of the living

worm of *Ps. occelata*. The author reexmained the relations between the differentiation of *Pr.* sp. and *Ps. occelata* in her experimental investigation already as mentioned. The gonozooid appeared from the stolon independently on the worm body of *Ps. occelata*. The polyp (gastrozooid) differentiated only when the stolon kept close contact with the worm body. When the differentiation of the stolon kept close contact with the tentacle, trunk and the tail of the worm body respectively, it was found that the polyp differentiated when it keeps close contact with every part of the worm body except the tentacle. This was recognized by that the polyp was able to differentiate when the stolon was transplanted on the cut part of the posterior end of the tube with the tail of the worm where the stolon could keep close contact with the regenerated tail end of the worm. Transplantation of the stolon on the worm body directly was tried, and the differentiation of the polyp from the transplanted stolon was observed. There-from it was inferred that the factor which is necessary for the differentiation of the polyp is the substance secreted from the worm body, and especially that the substance for tube formation secreted and mixing with sea-water for harding of the tube is an important factor.

From the results of the investigations of Hirai (1960) and of the present paper, it is inferred that the differentiation of the hydrozoan *Pr.* sp. greatly depends on the tube formation substance which is secreted from the worm, and that the normal pattern of the colony and life of *Pr.* sp. depends on the tube in which the living worm or the body of *Ps. occelata* is kept.

From the stolon transplanted on the young larva which passed eight days after spawning, the polyp was differentiated. From this fact, it was considered that there is a close relation between the life cycles of *Ps. occelata* and *Pr.* sp. in the field.

SUMMARY

A hydrozoan *Proboscidactyla* sp. lives commensal upon the tube of *Pseudopotamilla occelata*. From the results of Hirai (1960) and the present experimental investigations it was found that the polyp (gastrozooid) of *Pr.* sp. was differentiated when the stolon kept close contact with every part of the worm body of *Ps. occelata* except the tentacle. It is inferred that the tube formation substance which is secreted from the worm body was the main factor for the differentiation of the polyp of *Pr. sp.*, and it is considered that the life-cycle with the normal pattern of the colony and the differentiation of *Pr. sp.* were kept only on the tube of *Ps. occelata* with the living worm body in the tube.

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Explanation of Plate II

Pl. I. Life cycle of *Proboscidactyla* sp.

1. Entangling association of *Pseudopotamilla occelata*.
2. Polyps of *Pr.* sp. at the edge of the worm tube of a living *Pseudopotamilla occelata*.
3. Polyps with two tentacles arranged dorso-ventrally to the axis of the worm tube. The ventral side of the polyp is directed toward the inner part of the tube.
4. The worm removed from its tube.
5. Medusae buds on the middle part of gonozooid.
6. A young medusa which just escaped from a gonozooid. Medusa escapes from the gonozooid about 10–12 days after the bud appeared. The umbrella is about 0.5 mm in height and about 0.7 mm in width.